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In silico analysis of cytochrome p450 genes involved in the metabolism of diterpenes in *Coffea*

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Brazil is the largest world producer and exporter of coffee, being also the second largest consumer market. Among the main goals of coffee breeders, studies aiming the improvement of cup quality and plant tolerance to biotic and abiotic stresses have extreme importance. Beverage nutraceutical properties and plant defense mechanisms are directly linked to diterpenes present in the lipid fraction of coffee beans, such as cafestol (Caf) and caveol (Cav). Many members of P 450 gene family are involved in plant secondary metabolism, including diterpenes synthesis. In order to depict biochemical and genetic aspects of diterpenes byosinthesis, we did an in silico characterization of p450 gene family in Coffea spp., and we also quantified Caf and Cav in coffee fruit tissues for further gene expression studies involving diterpens metabolism. Using keyword and Blast search, 1396 ESTs related to Cyt p450 were selected from the Brazilian Coffee Genome Project (http://www.lge.ibi.unicamp.br/cafe). After assembling, we observed 157 putative unigenes, distributed in 92 contigs and 65 singlets. The contigs were analyzed using BLAST X versus public sequences databases (GenBank and Harvest Coffea), confirming their identity to 91 Cyt P450 genes. Expression profiles were inferred by electronic Northern blot of all contigs, allowing the selection of 7 candidate genes for transcriptional analysis based in fruit cDNA library expression. Caf and Cav were measured using HPLC in two different fruit developmental stages: 90 DAF (Days After Flowering) vs 120 DAF and in fruits (120 DAF) treated with 2µM methyl Jasmonate (MJ). Fruits at 120 DAF had an increase of 42% in Cav and 19% in Caf levels in relation to 90DAF fruits. MJ treatment resulted in samples with an average increase of 18% of Cav and 35% of Caf. RNAs were extracted from these samples for future transcriptional analyses. This study establish a platform for expression analysis of cyt P450 candidate genes in RNA samples from tissues with contrasting accumulation of Cav and Caf.

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